Phenetic distinction between the dwarf yellow water-lilies: *Nuphar microphylla* and *N. pumila* (Nymphaeaceae)

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Abstract: *Nuphar pumila* (Timm) DC. and *Nuphar microphylla* (Pers.) Fern. are morphologically similar yellow waterlily species that are often regarded as conspecific or recognized as varieties of *Nuphar lutea* (L.) Sm. This phenetic study analyzes 18 features of vegetative, floral, and fruit morphology scored from North American populations of *N. microphylla* and Eurasian populations of *N. pumila*. Morphological similarities among specimens were assessed by univariate statistics, clustering by the unweighted pair group method using arithmetic averages (UPGMA), and ordination by principal components analyses. Means for 17 of the 18 quantitative variables differed significantly between operational taxonomic units (OTUs) of the two species, and an UPGMA phenogram provided good separation of OTUs. Principal components analysis also provided reasonable separation of OTUs, indicating leaf and fruit characters as strong distinguishing features. Multivariate analyses indicate two similar, yet distinct, morphological entities. When coupled with qualitative features, geographical barriers, and putative physiological barriers, morphometric data support the taxonomic recognition of two closely related species. Both species are most closely related to the Japanese endemic *Nuphar japonica* DC.

Key words: Nuphar, Nymphaeaceae, water-lily, taxonomy, phenetics.

Résumé : Le *Nuphar pumila* (Timm) DC. et le *Nuphar microphylla* (Pers.) Fern. sont deux espèces de lys d'eau jaunes et morphologiquement similaires qu'on considère souvent comme conspécifiques ou encore comme variétés du *Nuphar lutea* (L.) Sm. Dans cette étude phénétique, les auteurs analysent 18 caractéristiques de la morphologie végétative, florale et du fruit, obtenues à partir de populations nord-américaines du *N. microphylla* et de populations eurasiennes du *N. pumila*. Les similitudes morphologiques entre spécimens ont été évaluées en utilisant l'analyse statistique univariée, les regroupant par la méthode de groupe de paires non-pondérées en utilisant la moyenne mathématique (UPGMA), et l'ordination par analyse en composantes principales. Les moyennes pour 17 des 18 variables quantitatives diffèrent significativement entre les unités taxonomiques opérationnelles (OTUs) des deux espèces, et un phénogramme UPGMA permet de bien séparer les OTUs. L'analyse en composantes principales fournit également une séparation raisonnable des OTUs, ce qui indique que les caractères des feuilles et des fruits sont des caractéristiques fortement distinctives. Les analyses multivariées indiquent deux entités morphologiques similaires mais également distinctes. Les données morphométriques, couplées aux caractéristiques qualitatives, géographiques et physiologiques putatives, supportent la reconnaissance systématique de deux espèces étroitement reliées. Les deux espèces sont fortement reliées à l'espèce japonaise endémique, *Nuphar japonica* DC.

Mots clés : Nuphar, Nympheaceae, lys d'eau, taxonomie, phénétique.

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Introduction

Nuphar Sm. (Nymphaeaceae) is a common and conspicuous component of the freshwater flora throughout most of the Northern Hemisphere. Overall, the genus has received ample systematic attention over the past century. Yet one persistent taxonomic controversy within *Nuphar* has concerned the distinction of the North American and the Eurasian dwarf yellow water-lilies, *Nuphar microphylla* (Pers.) Fern., and *Nuphar pumila* (Timm) DC., respectively. Both these taxa are clearly differentiated within the genus being

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diminutive in overall form, and thus they are regarded commonly as "dwarfs." Although the ranges of *N. microphylla* and *N. pumila* do not overlap, they are morphologically similar in several respects. Both possess five sepals, fruits with elongated necks, lobed stigmatic disks, slender petioles, and floating leaves with relatively deep sinuses. The two taxa have been distinguished primarily by anther length, stigmatic disk color, and geography (Hooker 1835*b*; Morong 1886).

The *Nuphar* dwarfs have interested students of the genus from both taxonomic (Hara 1951) and horticultural standpoints (Aiton 1811). Their unique morphology (Miller and Standley 1912; Heslop-Harrison 1953), biogeographical history (Heslop-Harrison 1955), and documented involvement in interspecific hybridization (Heslop-Harrison 1953; Padgett et al. 1998) have been subjects of examination. In particular, interests in the dwarfs have grown over the rarity of populations in North America. Historically, *N. microphylla* was much more common in northeastern North America. These plants are now legally protected or tracked throughout much of their range (Beaman et al. 1985; Anonymous 1992, 1997*a*, 1997*b*, 1998) and considered extirpated from some areas (Anonymous 1997*c*).

Taxonomic history

The Eurasian dwarf plants were first recognized as a distinct entity within the genus by Timm (1795) as Nymphaea lutea var. pumila Timm. The North American representatives were later recognized by Michaux (1803) as Nymphaea lutea var. kalmiana Michx. (Note that the name Nymphaea L. at that time included the yellow water-lilies, now known as Nuphar). Both taxa eventually were elevated to species level, with the epithet "microphylla" applied to the North American plants at specific rank, having priority over "kalmiana" (see Miller and Standley 1912). Hooker (1821, 1835a), Gray (1895), Heslop-Harrison (1955), Hultén (1971), and Voss (1985) treated both the Eurasian and North American dwarf yellow water-lilies as a single species, N. pumila. Similarly, Beal (1956) combined the two as a single taxon but treated it at the subspecific rank, as N. lutea subsp. pumila (Timm) Beal. Others, in contrast, have accepted them as two distinct species (Lawson 1888; Caspary 1891; Miller and Standley 1912; Gleason and Cronquist 1991; Wiersema and Hellquist 1997).

In 1993, fieldwork was conducted in south-central Siberia, Russia, where *N. pumila* was collected. During the same field season, *N. microphylla* was collected within the Lake Champlain Valley of Vermont, U.S.A. Initial observations of the North American plants indicated they were much smaller overall, had red stigmatic disks, and more globose fruits than the Siberian *N. pumila*. These field studies strongly indicated a distinctness between the two dwarf *Nuphar* species and suggested a need for more critical morphological comparisons.

The unclear taxonomic delimitation of the dwarf taxa justifies the re-evaluation of their status. In particular, the delimitation of *N. microphylla* must be addressed to guide decisions concerning its conservation. To address these concerns, this study was carried out to expand the morphological analyses of *N. microphylla* and *N. pumila* to better characterize and clarify their phenetic relationship. Using quantitative methods, I sought to determine if clusters of morphologically distinguishable populations exist that correspond to these geographically discrete.

Materials and methods

This analysis applied the methods of numerical phenetics. Morphological characters were measured on 140 specimens drawn from 11 herbaria (BM, CONN, DAO, NASC, NCSC, NHA, NY, PH, S, UC, VT). Vouchers are listed in Appendix 1. Eighteen quantitative variables were measured, most of which have been used previously to distinguish *Nuphar* taxa, comprising seven vegetative, six floral, and five fruit features (Table 1). Leaf measurements were restricted to floating lamina and selected randomly among evidently mature (expanded) lamina of each specimen. A total of 77 operational taxonomic units (OTUs) of *N. microphylla*

and 63 OTUs of *N. pumila* were evaluated, selected from throughout the geographic and morphological range of each species.

Means and standard deviations were calculated for all variables using the SYSTAT (version 5.0) software package (Wilkinson 1990). Character means were compared between the two taxa using an analysis of variance (ANOVA) and a Tukey honestly significantly different (HSD) post hoc test. Phenetic analyses of all OTUs were carried out by the NTSYS-pc (version 1.80) computer package (Rohlf 1993). Unscorable data were treated as missing. Raw data for all statistically significant (p < 0.05) characters were standardized and similarity matrices (using average Manhattan distance) were generated. Clustering was performed using the unweighted pair-group method using arithmetic averages (UPGMA). Principal components analysis (PCA) was performed on the standardized similarity matrix of a reduced data set. The PCA data set included all characters found to be statistically significant (p <0.05) between the taxa (as in the UPGMA study) except variables L5 and G2, so as to avoid misleading correlations with variables L1 and G3, respectively (see Table 1).

Results

One-way ANOVA of the entire data set demonstrated that the means of 17 of the 18 characters (94%) were significantly different (0.05 level) between *N. microphylla* and *N. pumila* (Table 2). Anther length (F3), a character used historically as a key character to distinguish the two at the species level, was one of the significant characters. The ratio of leaf length to leaf width (L3) was the only character that did not show significance. For *N. pumila*, the means of 14 characters (77%) were larger than those of *N. microphylla* indicating that the former is more robust. The data show variability within and between the two entities with overlapping ranges for characters (Table 2).

The UPGMA phenogram based on the 17 significantly different characters, generated by average Manhattan distance, showed two reasonably discrete clusters. These clusters correspond to the separation of *N. pumila* and *N. microphylla* with some intermixing of OTUs (Fig. 1). The upper cluster of the phenogram was the largest, comprised largely (81%) of *N. pumila* OTUs. The other major cluster was comprised almost entirely (97%) of *N. microphylla* OTUs (Fig. 1).

A similar amount of phenetic structure is evident in PCA. By PCA, two clusters of OTUs are again distinguishable with some interdigitation (Fig. 2). The PCA accounted for 64.5% of the total variability in the first three components, 44.5, 11.5, and 8.5, respectively. Characters highly correlated with the first component were leaf length, leaf sinus length, and stigmatic disk (fruit) diameter (Table 3). Characters most highly correlated with the second component were flower width, leaf width, and fruit length. Characters most highly correlated with the third component were anther length, peduncle diameter, and fruit length (Table 3).

Discussion

Morphology and classification

Although taxonomic opinions on the distinctness of these taxa (regardless of the rank employed) have been divided, the key characters utilized to distinguish them have likewise been inconsistent. Maintaining separate species, Morong (1886) and Miller and Standley (1912) saw differences in

Designation	Character description
Leaves	
LI	Length of leaf (cm)
L2	Width of leaf (cm)
L3	Ratio between leaf length and leaf width (L1/L2)
L4	Length of leaf sinus (cm)
L5	Ratio between sinus length and leaf length (L4/L1)
L6	No. of lateral leaf veins
L7	Diameter of petiole at 5 cm from base of blade (mm)
Flower	
F1	Length of mature flower (cm)
F2	Width of mature flower (cm)
F3	Length of anther (mm)
F4	Diameter of stigmatic disk (mm)
F5	No. of stigmatic rays
F6	Diameter of peduncle at 5 cm from base of flower (mm)
Fruit	
Gl	Length of mature fruit (cm)
G2	Width of mature fruit (cm)
G3	Ratio between fruit length and fruit width $(G1/G2)$
G4	Diameter of constriction below stigmatic disc (mm)
G5	Diameter of stigmatic disk (mm)

Table 1. Morphological characters scored for analysis.

Note: Leaf characters were scored from exposed (floating) leaves.

Table 2. Comparison of Nuphar microphylla and N. pumila for the 18 morphological characters listed in Table 1.

		N. microphylla				N. pumila				
Character	n	Minimum	Mean	Maximum	SD	n	Minimum	Mean	Maximum	SD
Ll	55	4.20	7.19	13.00	1.95	56	6.80	10.88	15.40	1.89
L2	55	3.20	5.20	8.00	1.16	56	5.50	7.98	10.80	1.22
L3	55	1.10	1.37	1.60	0.13	56	1.09	1.36	1.72	0.11
L4	55	1.30	2.95	4.80	0.73	55	3.00	4.37	6.30	0.74
L5	55	0.39	0.56	0.73	0.06	55	0.32	0.40	0.63	0.04
L6	51	5.00	8.62	15.00	2.08	52	10.00	12.69	17.00	1.87
L7	45	1.00	1.29	2.50	0.43	38	1.00	2.15	5.00	0.63
F1	29	0.90	1.26	1.90	0.23	26	1.00	1.56	2.70	0.35
F2	29	1.20	1.63	2.10	0.27	26	1.30	1.88	2.30	0.25
F3	28	1.00	2.01	3.00	0.63	36	1.00	1.48	2.50	0.40
F4	26	2.50	4.03	6.00	0.84	35	3.50	6.04	10.00	1.37
F5	29	6.00	8.06	11.00	1.22	38	8.00	10.57	13.00	1.28
F6	30	1.50	2.60	4.00	0.63	39	2.50	3.69	5.50	0.71
G1	21	1.00	1.61	2.50	0.31	31	1.50	2.26	3.00	0.41
<i>G2</i>	21	0.90	1.45	2.10	0.28	31	0.90	1.36	1.90	0.27
G3	21	0.79	1.13	1.56	0.19	31	1.21	1.69	2.31	0.32
G4	20	1.00	2.17	3.00	0.49	31	2.50	3.21	4.00	0.47
G5	15	2.50	3.46	6.00	0.71	22	4.00	5.43	7.50	0.93

Note: Sample size (*n*), mean values, standard deviation (SD), and minimum and maximum values are given. Both species differ significantly (p < 0.05) for every character mean except L3 (leaf length/width ratio).

stamen morphology, yet admitted overall morphological similarities. Hara (1951) stated that *N. microphylla* differed from *N. pumila* in the size and shape of fruits, size of seeds, and presence of stamens on mature fruits. On the other hand, Beal (1956) asserted that the plants were so similar in size and shape of leaves, flowers, sepals, fruits, seeds and rhizomes that he placed both in one taxon, *N. luteum* ssp. *pumilum*.

The numerical evaluation of morphological characters of *N. microphylla* and *N. pumila* reveals that OTUs of the respective species differ in leaf, flower, and fruit features. Means for 17 of the 18 characters examined were significantly different, with features of *N. pumila* larger overall (Table 2). The overlap of character ranges is indicative of the variability and close phenetic similarity of the two species, presumably the reason for disagreement among various

Fig. 1. UPGMA phenogram (average Manhattan distance) of 140 OTUs based on morphological variables of *Nuphar pumila* and *N. microphylla*. Circles are individual OTUs of *N. microphylla* assigned to the cluster of *N. pumila* OTUs. Squares are OTUs of *N. pumila* assigned to the *N. microphylla* cluster.



taxonomic treatments. While the UPGMA clustering provided incomplete separation of *N. microphylla* and *N. pu-mila* OTUs, two clusters are discernible (Fig. 1).

The multivariate analysis of the data likewise provided distinct, yet closely positioned, clusters of OTUs with some occasional overlap (Fig. 2). The inability of these latter analyses to separate completely the OTUs of *N. microphylla* and *N. pumila* also reflects their morphological similarity. The PCA revealed that leaf length, sinus length, and stigmatic disk diameter (fruit) are the most effective characters for separating *N. microphylla* and *N. pumila*, with fruit size and leaf width of secondary importance. These characters have not been considered previously as diagnostic. Anther length, a character most commonly used to separate the two as distinct taxa, was also found to be diagnostic, although not as greatly as the former characters. Thus, the statistical analyses corroborate that North American dwarf yellow water lilies are morphologically different from those of Eurasia.

The morphological separation of *N. microphylla* from *N. pumila* is also evident in many features (quantitative and qualitative) that were excluded from the formal numerical





Table 3. Correlations of variables (see Table 2) used in principal components analysis and loadings in the first three principal axes.

Variables	Ι	II	III
Ll	0.842 32 ^a	0.201 07	0.114 61
L2	0.380 44	$0.623~76^{b}$	0.240 39
L4	$0.825\ 51^b$	0.192 30	0.189 49
L6	0.746 52	0.143 08	-0.144 23
L7	0.663 05	0.221 18	0.353 50
F1	0.561 66	0.283 06	-0.338 27
F2	0.488 52	0.639 49 ^a	-0.223 96
F3	-0.309 56	0.160 81	$-0.663 \ 12^{a}$
F4	0.636 55	-0.411 14	-0.007 27
F5	0.747 21	-0.052 12	0.054 98
F6	0.538 19	-0.413 69	$0.395 \ 87^b$
G1	0.679 56	-0.441 09°	$-0.392\ 10^{c}$
G3	0.737 15	-0.325 76	-0.149 37
G4	0.757 89	-0.051 45	-0.294 51
G5	$0.819~77^{c}$	-0.168 71	-0.032 62

Note: Superscripts identify variables with the three highest correlations to each axis and identify the variables contributing most.

analyses (Table 4). The most striking feature distinguishing the two groups is the coloration of the stigmatic disks (Table 4). The dark red stigmatic disk of *N. microphylla* is exceptional within the genus. There are, however, reports of red-tinged stigmatic disks in east Asian populations of *N. pumila* (Hara 1951; Beal 1956). The data presented in Table 4 are consistent with results from the statistical analyses of other features in demonstrating the features of *N. pumila* to be larger than those of *N. microphylla*. While degree of pubescence on the undersides of floating leaves has been reported to differ between *N. microphylla* and *N. pumila*, being more dense in the latter (Morong 1886; Beal 1956), the degree of pubescence was found to be an extremely variable feature in these taxa and in *Nuphar* as a whole (Padgett 1997).

The geographical distributions of *N. microphylla* and *N. pumila* are well defined and strictly allopatric. *Nuphar*

Character	N. microphylla	N. pumila
Sepal shape	Obovate or elliptical	Narrowly spathulate to ovate
Sepal length (mm)	About 10	16–29
Sepal width (mm)	6–8	9–16
Petal number	7–10	9–13
Stamen at fruit maturity	Deciduous	Persistent
Stigmatic disk color	Dark red	Yellow (rarely reddened)
Fruit shape	Ovoid	Flagon shaped
Fruit color	Burnt carmine tinged	Green
Seed color	Yellow brown	Greenish brown

Table 4. A comparison of select qualitative and quantitative characters of *Nuphar microphylla* and *N. pumila* offered by Miller and Standley (1912) and Heslop-Harrison (1955), respectively.

Fig. 3. Geographical distribution of Nuphar microphylla.



microphylla is restricted to northeastern North America, extending from southern Manitoba east to Nova Scotia, south to eastern Wisconsin and New Jersey (Fig. 3). *Nuphar pumila* is strictly Eurasian, extending from northern Europe east to the Kamchatka Peninsula (Russia) and Japan, south to southern China and Switzerland in Europe (Fig. 4). Both occupy similar habitats of deeper freshwater ponds, lakes, and sluggish watercourses. However, in New England, *N. microphylla* reportedly inhabits neutral to alkaline waters (Hellquist and Crow 1984), while *N. pumila* of Europe is found in circumneutral to acid waters (Heslop-Harrison 1955).

There are two published accounts that offer insight into genetic barriers between *N. microphylla* and *N. pumila* (Morong 1886; Fletcher 1883). However, both of these accounts lack empirical data. Fletcher (1883) recounted artificial crosses made between *N. microphylla* and *N. pumila* by Robert Caspary. According to Fletcher (1883), Caspary concluded from numerous experiments, that both species were distinct, although morphologically similar. Likewise, Morong (1886) reported a "physiological difference" between *N. microphylla* and *N. pumila* when the two were crossed artificially. A preliminary survey of randomly amplified DNA

(RAPD) revealed several unique loci for both *N. pumila* and *N. microphylla*, yet showed a large number of shared markers between them (D. Padgett, unpublished data). Distinctions between *N. microphylla* and *N. pumila* at the molecular level need further investigation.

Results of this study contradict the conclusion of Beal (1956) and others that the differences between the North American *N. microphylla* and European *N. pumila* are weak. Differences based on the characters utilized in the numerical analysis, combined with additional differences in morphological features (Table 4), indicate clear morphological divergence among the dwarf yellow water-lilies. Furthermore, these differences are discernible in field observations. In my opinion, these two groups of OTUs are sufficiently distinct from each other to merit continued recognition at species level. The phenetic evidence, combined with the geographical isolation and putative physiological barriers between the two taxa, reinforces the taxonomic recognition of these entities as two distinct, but closely related, species.

Information gleaned from recent phylogenetic studies of the genus precludes any attempt to treat either entity at a subspecific level under *Nuphar lutea* (L.) Sm. Reconstructed

Fig. 4. Geographical distribution of Nuphar pumila.



relationships within *Nuphar* fail to corroborate Beal's (1955, 1956) hypothesis of a wide-ranging, polymorphic species (as *N. lutea*) in the Old and New Worlds that embraced, among other taxa, *N. microphylla* and *N. pumila* (Padgett 1997; Padgett et al. 1996). The Eurasian *N. lutea* is morphologically distinct from the dwarf taxa and only remotely related to them.

Evolution and biogeography

In light of this information, it is worthwhile to interpret the phylogenetic history of these dwarf taxa. Cladistic analysis of morphological and molecular data (chloroplast and nuclear DNA sequences) of the entire genus indicates two major groups of species (Padgett 1996, 1997; Padgett et al. 1996). These species groups correspond biogeographically to an Old World – New World divergence. These data indicate that both *N. pumila* and *N. microphylla* have affinities with the "Old World" lineage. The dwarf taxa represent a terminal clade within this lineage, both derived from a common ancestor (Padgett 1996, 1997). The species most closely related to *N. pumila* and *N. microphylla* is *Nuphar japonica* DC. Endemic to Japan, *N. japonica* is an overall larger, emergent-leaved species and the only other species to exhibit a lobed stigmatic disk (Padgett 1997).

The phylogenetic alliance of the northeastern North American *N. microphylla* to a group confined to Eurasia is interesting biogeographically. It can be hypothesized that *N. microphylla* evolved from a *N. pumila*-like ancestor following the isolation and divergence of the two larger lineages. Without further information, the time and geographic origin (western Europe or eastern Asia) of the ancestor of *N. microphylla* can only be speculated. The relatively low molecular divergence and similar morphology between *N. microphylla* and *N. pumila* would suggest a rather recent divergence. Interestingly, as the sole "Old World" representative

in North America, the present range of N. microphylla appears to be contracting, and it is now rare throughout much of its range (Padgett 1997). In the context of morphological variation, populations of N. microphylla are divergent and strongly isolated, making their conservation a priority.

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Appendix 1.

Voucher specimens of *Nuphar microphylla* and *N. pumila* used in morphological analyses.

N. pumila

AUSTRIA: Styria, Steyrmark, 26 July 1922, *Rechinger s.n.* (BM).

CHINA: Kweichow, border of Kwangsi, *Tsiang 7422* (S); Mandshuria, austro-orientalis, 1870 (BM), near stn. Tmemno, *Litvinov 2259* (NY), near stn. Chingis-Khan, *Litvinov 3407* (NY).

DENMARK: Bornholm, Sø i Rø Plantage, 25 June 1951, Dahl s.n. (BM).

FINLAND: Pohjois-Karjala, Kiiminki, 24 July 1963, Langstedt s.n. (UC); Kajaani, Siikalahti, 6 Aug. 1969, Heikkinen s.n. (S); Lapponica, Jomppala Lake, Jordan 247 (BM); Lkem., Kittila, Alava et al. 4454 (UC); Satakunta, Ylöjärvi, 7 Aug. 1911, Florström s.n. (S); Savonia borealis, par. Maaninka, 4 Aug. 1918, Kyyhkynen s.n. (UC), par. Maaninka, Kyyhkynen 1170 (UC); Karelia australis, par Vehkalahti, 6 July 1960, Fagerstrom s.n. (UC).

FRANCE: Cantal: Lac du Tayer, July 1902, Charbounel s.n. (BM), July 1902, Charbonnel s.n. (BM); Lac de Chamberdaze, 8 Aug. 1903, Chassaspre s.n. (BM); Vosges: Lac de Retournemer, Deseglise 141 (BM); Lac de Gerardmer, Anthelme 5360 (BM), 24 July 1867, Caspary s.n. (US); Machey, Retz 7100 (BM); Lac du Vosges (BM); Remiremont, 26 Aug. 1867, Caspary s.n. (BM).

GERMANY: Hesse, Langen, 27 Aug. 1884, *Caspary s.n.* (BM); Neustadt, 27 Aug. 1884, *Caspary s.n.* (BM).

JAPAN: Hokkaido, Nemuro, 10 July 1959, *Furuse s.n.* (S). LATVIA: Riga, *Kupffer 12500* (DAO).

NORWAY: Arkershus, 6 Aug. 1894, *Dyring s.n.* (S); Oppland, Snertingdal, 1 Aug. 1938, *Holmboe s.n.* (BM); Trondheim, 1 Aug. 1890, *Lilliesleold s.n.* (S).

POLAND: Pomorze, Chojnicki, Greinert 321 (BM).

RUSSIA: Kamchatka Peninsula: Bolsheredsk, 17 Aug. 1921, Hultén 2890 (S); Paratunka, 1831, Rieder s.n. (S),

1921, Hullen 2890 (S); Paratulika, 1851, Kleuer S.n. (S), Hultén 3645 (S).

SCOTLAND: Aberdeen, Lock Kinnord, 2 Aug. 1879, Lowax s.n. (BM), 19 July 1946, Taylor s.n. (BM); Argyle, near Kingshouse, 19 July 1889, Marshall s.n. (BM); Caithness Co., Loch of Winless, Aug. 1885, Grant s.n. (BM); Glasgow, Bachhouse s.n. (BM); Inverness Co.: Aviemore, Druce 315 (BM); Wilmott 36715 (BM); Perth, Loch Lubnaig, 11 July 1936, Lansley s.n. (BM); Stirlingshire Co., Loch Lubnaig, Foggitt 54 (BM); Loch Bardowie, McKay 38 (BM).

SWEDEN: Dalecarlia, Mora, 22 July 1886, Olsson et al. s.n. (BM); Fryken, 15 July 1926, Svensson s.n. (S); Jämtland, Hammerdals, 19 July 1927, Lange s.n. (S); Kopparberg, Orsa, Aug 1897, Egerström s.n. (S), 30 July 1926, Johanson s.n. (S); Mjörn, July 1895, Alströmer s.n. (BM); Norbotten: Pitea, 4 Aug. 1908, Marklund s.n. (S), 1 Jul 1869, Lundström s.n. (S); Tarendo parish, Koivuniemi, Alm 3648 (UC), Saittajarvi, Alm 3736 (BM); Östergötland: 1838, *Ekenoth s.n.* (S); Herresater, *Meauden s.n.* (S); Atvidaberg, 10 Aug. 1904, *Hulphers s.n.* (S); Smiland, 20 June 1921, *Trolander s.n.* (S); Västerbotten, Bygdea, 8 Aug. 1906, *Fahlander s.n.* (BM).

WALES: Merioneth, 9 Aug. 1886, Ley s.n. (BM).

N. microphylla CANADA: Manitoba: Parker Bog, Parker 85-775 (DAO). New Brunswick: Northumberland Co., Webster & Fielding 178 (DAO); Restigauche Co., McDougall Lake, Roberts & Drury 63-1882 (DAO); St. John's River, Hay 98 (BM). Ontario: Corry Lake, Breitung 6818 (MT); Glengarry Co., w. of Alexandria, Dore 21444 (DAO); Kenora District, Lake of the Woods, Macins 39-67 (DAO); Lac James, Chalk River, Vladykon v-3 (DAO); Renfrew Co., Westmeath, Darbyshire & Dore 1639 (DAO); Buckanan, Ottawa River, Breitung 7060 (DAO); Thunder Bay District, Black Sturgeon Lake, Garton 12532 (DAO). Quebec: Gaspé Co., Baie des Chaleurs, Marie-Victorin et al. 44324A (MT); Iberville Co., Henryville, Adrien 2092 (MT); Vaudreuil Co., Rigaud, Roy 3343 (DAO); Sainte-Rose, Laval, Marie-Victorin & Rolland-Germain 44307 (DAO); St. Eustache, Victorin s.n. (UC).

U.S.A.: Connecticut: New Haven Co., Milford, *Eames* 1798 (CONN). Maine: Androscoggin Co.: Auburn, 13 Jul 1875 (NHA); Aroostook Co.: Round Pond T13, R12, *Lawe* 19445 (NHA); St John River, *Fernald s.n.* (CONN); St. Francis, *Fernald 10* (NHA), *Evans 16001* (NHA); Littleton-Houlton line, *Hellquist 13842* (NASC); Presque Isle, *Chamberlain 2126* (UC), *Hellquist et al. 13873* (NASC); Washburn, *Hellquist 5971* (NASC), *Crow 2941* (NHA); Leanwell, Girard Pond, *Norton 8275* (NHA); Houlton, *Crow*

et al. 2932 (NHA); Oxford Co.: Gilead, Moore 1119 (UC); Somerset Co.: Township VI, St. John & Nichols 2291 (US); Washington Co.: Edmunds, Pike et al. s.n. (NHA); York Co.: Alfred, Cleonique-Joseph 6165 (MT). Massachusetts: Berkshire Co.: Sheffield, Weatherbee 3743 (NHA); Hampden Co.: Holyoke, Lumsden s.n. (UC); Middlesex Co.: Concord, Sudbury River, Worthen s.n. (US). New York: Cortland Co.: Willow Grove, Wiegand 6430 (NCSC); Herkimer Co.: Gray, House s.n. (US); McDonough, Coville s.n. (US); Saratoga Co.: Coveville, Muenscher & Lindsey 3316 (UC); St. Lawrence Co.: Canton, Phelps s.n. (NCSC); Lonesome Bay, Muenscher & Maguire 2254 (UC); Ulster Co.: Stoney Ridge, Manning s.n. (FLAS); Washington Co.: Whitehall, Lake Champlain, Carpenter s.n. (VT). Vermont: Addison Co.: Addison, Wodehouse s.n. (VT); Ferrisburg, Hellquist 5665 (NASC), Lewis Creek, Padgett 480 (NHA), Little Otter Creek, 16 Aug. 1896 (VT), 16 Aug. 1896, Grout s.n. (VT), Eggleston 2543 (VT); Hancock, Dutton s.n. (VT), Lost Pleiad Pond, 18 July 1879 (VT); Caledonia Co.: East Barnet, Blanchard s.n. (UC); Danville, Grout s.n. (VT); Chittenden Co.: Burlington, Flynn s.n. (VT); Shelburne, 24 July 1862, Pringle s.n. (VT), 15 July 1878, Pringle s.n. (VT), La Platte River, Padgett 482 (NHA); Colchester, Zika 1760 (VT), Flynn s.n. (VT); Franklin Co.: Highgate, Jesup s.n. (NHA); Orleans Co.: Barton, Crystal Lake, Hellquist 5082 (NASC); Irasburg, Hellquist 2766 (NASC), Hellquist 2765 (NASC); Washington Co.: East Montpelier, Tower 6891 (VT); 23 Feb. 1909, Pringle s.n. (UC).